STABLE HETERODIMERIC ANTIBODY DESIGN WITH MUTATIONS IN THE FC DOMAIN

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 15/411,799, filed Jan. 20, 2017, which is a divisional of U.S. application Ser. No. 13/668,098, filed Nov. 2, 2012, now U.S. Pat. No. 9,574,010, which claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 61/556,090, filed Nov. 4, 2011; U.S. Provisional Patent Application No. 61/557,262, filed Nov. 8, 2011; and U.S. Provisional Patent Application No. 61/645,547 filed May 10, 2012, each of which is incorporated herein by reference in its entirety.

INTRODUCTION

Field of the Invention

[0002] The present disclosure generally provides polypeptide heterodimers, compositions thereof, and methods for making and using such polypeptide heterodimers. More specifically, the present invention relates to thermo-stable multispecific, including bispecific, antibodies comprising a heterodimeric Fc domain.

Background of the Invention

[0003] Bispecific therapeutics are antibody-based molecules that can simultaneously bind two separate and distinct targets or different epitopes of the same antigen. Bispecific antibodies are comprised of the immunoglobulin domain based entities and try to structurally and functionally mimic components of the antibody molecule. One use of bispecific antibodies has been to redirect cytotoxic immune effector cells for enhanced killing of tumor cells, such as by antibody dependent cellular cytotoxicity (ADCC). In this context, one arm of the bispecific antibody binds an antigen on the tumor cell, and the other binds a determinant expressed on effector cells. By cross-linking tumor and effector cells, the bispecific antibody not only brings the effector cells within the proximity of the tumor cells but also simultaneously triggers their activation, leading to effective tumor cell-killing. Bispecific antibodies have also been used to enrich chemo- or radiotherapeutic agents in tumor tissues to minimize detrimental effects to normal tissue. In this setting, one arm of the bispecific antibody binds an antigen expressed on the cell targeted for destruction, and the other arm delivers a chemotherapeutic drug, radioisotope, or toxin. Going beyond bispecifics, there is a need for protein therapeutics to achieve their efficacies by targeting multiple modalities concurrently. Such complex and novel biological effects can be obtained with protein therapeutics by designing multi-target binding and multi-functional aspects into the protein.

[0004] A robust scaffold that provides a framework to fuse other functional war-heads or target protein binding domains in order to design these multifunctional and multi-target binding therapeutics is required. Ideally, the scaffold should not only provide the framework but also make available a number of other therapeutically relevant and valuable features to the designed therapeutic. A major obstacle in the general development of antibody-based bispecific and multifunctional therapeutics has been the difficulty of producing materials of sufficient quality and quantity for both preclinical and clinical studies. There remains a need in the art for polypeptide constructs that comprise single variable domains as the protein binding domains that are linked to a variant Fc region, said variant Fc comprising CH3 domains, which have been modified to select for heterodimers with an increased stability and purity.

SUMMARY OF THE INVENTION

[0005] There is provided according to one aspect of the invention an isolated heteromultimer Fc construct comprising a modified heterodimeric CH3 domain, said modified CH3 domain comprising: a first modified CH3 domain polypeptide comprising at least three amino acid modifications as compared to a wild-type CH3 domain polypeptide, and a second modified CH3 domain polypeptide comprising at least three amino acid modifications as compared to a wild-type CH3 domain polypeptide; wherein at least one of said first and second CH3 domain polypeptides comprises an amino acid modification of K392J wherein J is selected from L, I or an amino acid with a side chain volume not substantially larger than the side chain volume of K; wherein said first and second modified CH3 domain polypeptides preferentially form a heterodimeric CH3 domain with a melting temperature (Tm) of at least about 74° C. and a purity of at least 95%; and wherein at least one amino acid modification is not of an amino acid which is at the interface between said first and said second CH3 domain polypeptides. In certain embodiments is a heteromultimer Fc construct described herein, comprising at least one T350X modification, wherein X is a natural or non-natural amino acid selected from valine, isoleucine, leucine, methionine, and derivatives or variants thereof. In some embodiments is an isolated heteromultimer Fc construct described herein, comprising at least one T350V modification. In an embodiment is an isolated heteromultimer Fc construct described herein, wherein the modified CH3 domain has a melting temperature (Tm) of at least about 75° C. or greater. In an embodiment is the isolated heteromultimer Fc construct described herein, wherein the modified CH3 domain has a Tm of about 70° C. or greater. In certain embodiments, the modified CH3 domain has a Tm of about 80° C. or greater. Provided in certain embodiments is an isolated heteromultimer Fc construct described herein, wherein at least one CH3 domain polypeptide is a modified CH3 domain polypeptide comprising an amino acid modification of at least one of L351, F405, and Y407. In some embodiments is an isolated heteromultimer Fc construct, wherein at least one CH3 domain polypeptide is a modified CH3 domain polypeptide further comprising an amino acid modification of T366. In certain embodiments is an isolated heteromultimer Fc construct described herein, wherein the first CH3 domain polypeptide is a modified CH3 domain polypeptide comprising amino acid modifications at positions L351, F405, and Y407, and the second CH3 domain polypeptide is a modified CH3 domain polypeptide comprising amino acid modifications at positions T366, K392, and T394. In an embodiment is the isolated heteromultimer Fc construct described herein, said first CH3 domain polypeptide comprising amino acid modifications L351Y, F405A, and Y407V, and said second CH3 domain polypeptide comprising amino acid modifications T366L, K392M, and T394W. In some embodiments is the isolated heteromultimer Fc construct described herein, said first CH3 domain polypeptide comprising amino acid